

Extreme physiological tolerance leads the wide distribution of *Zaprionus indianus* (Diptera: Drosophilidae) in temperate world

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Abstract: During the last decades, *Zaprionus indianus*, a warm adapted drosophilid species has invaded and expanded its boundaries in the Indian subcontinent. *Z. indianus* has successfully invaded because of its high adaptive flexibility and extreme physiological tolerance. A comparison of developmental thresholds of *Z. indianus* (warm specialist) and *D. melanogaster* (cosmopolitan) during pre-adult and adult life stages at extreme temperatures showed significant difference in mortality and threshold temperature between both species. To test for variation in stress resistance and survival in preadult/adult stages over winter, *Z. indianus* populations were initiated from temperate and tropical sites in India. The hatchability of eggs and adult survival of these populations were monitored in field cages at a temperate location and under constant laboratory conditions, until all adults died. Total hatchability and survival showed significant difference with latitude, due to high hatchability and survival by temperate in northern populations as compared with southern populations. Egg to adult viability experiment indicated that eggs laid in low temperature conditions developed successfully to the adult stage when the temperature became moderate. This study is evident for climatic adaptation in immature stages and stress tolerance in adults that may provide seasonal protection to the species. When climate warming scenario was considered, *i. e.*, temperature increase of 0.6°C, a slight increase in temperature could cause a significant increment in population viability and a decrease in developmental duration. These results explain why *Z. indianus* can easily cross barriers and is able to adapt to new environment.

Key words: *Zaprionus indianus*; life stage; climatic adaptation; physiological tolerance; climatic warming

1 INTRODUCTION

One of the most persistent and recurrent problems for ecologist is why some animals are successful invader while others are not. Stress caused by climate change can affect insect populations in many ways. Overcoming environmental change is the first of several potential barriers determining whether a species becomes established, naturalizes and ultimately invades in a new locality (Pyšek and Richardson, 2006). The rate of spread and success in new environment of an invasive species sometimes depends largely on the ability to tolerate abiotic conditions (Kareiva *et al.*, 1996; Moyle and Light, 1996). The levels of winter survival are the primary determinant of future abundance in insects (Honek, 1989). Biological attributes of the species governs its range, invasion capacity and establishment. For invasive insect species in temperate regions, winter survival should be the determinant to reach great abundance after winter.

According to inter-governmental panel on climate change (IPCC, 2001), a global increase in

temperature of 0.6 degrees since mid nineteenth century is an alarming sign for biological system (Hughes, 2000; McCarthy *et al.*, 2001). The distribution of species is determined by biogeographic and climatic barriers (Rahel, 2002). Temperature is one of the most important factors that affect physiological performance, fitness and reproductive performance of the organism. Variation in resistance to thermal extremes across pre-adult life stages and studies specific to early life stage in *Drosophila* were studied (Krebs *et al.*, 1995) but not focused towards how climatic adaptation in immature stages helps the species to invade in new environment. Now-a-day's *Drosophila* species population has shown marked changes in distribution patterns, which may be due to the results of climate change, introduction of invasive species, shift in forest composition, *etc.* Global warming has led to shifts in species' geographical boundaries (Hughes, 2000; Rajpurohit *et al.*, 2008; Bradshaw and Holzapfel, 2008).

Zaprionus indianus is an excellent model for ecological and evolutionary studies focused on biological invasions. It is a native to Africa, and was first found in Americas in 1999 (Vilela, 1999). *Z. indianus* is a warm adapted species and the highest

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Received: 2012-07-12; Accepted: 2012-11-16

frequencies of this species have been registered during seasons having highest mean temperatures (Da Silva *et al.*, 2005). Previous studies have shown that *Z. indianus* has expanded its range in different environments, suggesting climatic niche shift (da Mata *et al.*, 2010). Based on biogeographical distribution of *Z. indianus* we have addressed the following questions: (a) how a highly warm specialist species has expanded its range even in temperate regions, (b) can we explain seasonal distribution on the basis of pre-adult stress tolerance and mortality, and (c) how 0.6-degree increase in temperature affects life history traits of a species. Here in this study we emphasize on the attributes that help one species in successful invasion but the other fails. For assessment of geographical variation, replicate population cages were initiated with adults originating from three different geographical localities. Climatic adaptations in immature stages were also studied. The main objective of this study is

to evaluate the winter survival strategy of *Z. indianus* in western Himalayas as a contribution to its successful invasion in temperate regions.

2 MATERIALS AND METHODS

2.1 Test flies

Z. indianus has been proven a successful invader in both tropical and temperate regions (Table 1). For the present study, flies were collected by net sweeping and bait trap methods from fruit orchards and fruit markets of three different localities: Solan (30.58°N) marginal locality for *Z. indianus* in north, Nagpur (21.06°N) in center and Mysore (12.18°N) in south on Indian subcontinent from March 2008 to February 2009. Temperatures in the field were monitored throughout the year. *Z. indianus* was separated from other species and were reared at 25°C (mid thermal range for *Z. indianus*).

Table 1 A survey of the published data on *Zaprionus indianus*

Country/Region	Subject	References
India	First record of <i>Z. indianus</i>	Gupta, 1970
South America	Recorded	Vilela, 1999
Brazil	Invasion record	Castro and Valente, 2001
Brazil	Invasion record	De Toni <i>et al.</i> , 2001
Uruguayan Territory	First record in southern localities of Uruguay	Goni <i>et al.</i> , 2001
Brazil	Invasion record	Tidon <i>et al.</i> , 2003
Brazil	Morphological variation and adaptation potential	Loh and Mathe, 2005
Panama and USA	Recorded as pest on fruits	Linde <i>et al.</i> , 2006
Brazil	Geographical variability	David <i>et al.</i> , 2006
Eastern Argentina	Distribution and host range	Lavagnino <i>et al.</i> , 2008
Africa, America and India	Climatic niche shift	da Matta, 2009
Brazil	Spread via transportation	Galego and Carareto, 2010

To test the genetic variability, wild caught flies from three regions with varying abiotic conditions (temperature and humidity) are used to establish twenty iso-female lines from each population.

2.2 Field and laboratory experiments

Simultaneous experiments were conducted in the field with fluctuating climatic conditions and in the laboratory under constant conditions. We investigated the performance of three populations of *Z. indianus* under low temperatures in the field by cage experiments and in laboratory by standard heat and cold resistance assays. We also checked the heat survival and cold mortality.

2.2.1 Developmental threshold experiment: For

estimating lower and upper developmental threshold of different stages (egg, larva, pupa and adult), we checked the viability of all stages at varying temperatures, both towards lower and upper. Lower and upper developmental thresholds were calculated for duration of non-dormant development under constant temperatures. For this experiment we compared *Z. indianus* (warm specialist) and *D. melanogaster* (cosmopolitan) species of *Drosophila*.

2.2.2 Field cage experiment: The first experiment was conducted in field cages. Field cages were established consisting of a clear plastic cylinder with fabric mesh windows to allow air circulation. Twenty pairs from each thirty iso-female (IF) lines were

used for cage experiments. Plastic chambers consisting of laboratory medium were placed inside each cage that served as an oviposition surface. Each cage is placed in a shady place and temperature inside and outside the cages were almost identical. Food chambers were exchanged regularly. Eggs were collected in replicates from laboratory reared flies were transferred to field and hatchability and total developmental duration was recorded. Eggs laid in the field were counted to measure hatchability, viability, developmental duration and longevity.

2.2.3 Experiments of variability in thermo tolerance traits:

Egg: Seven-day-old virgin flies were mated for egg collection at mid thermal range of *Z. indianus*. Forty replicates of eggs were collected every six hours on freshly prepared petri dishes. These plates were covered and randomly divided into two groups. The first group was exposed to low temperature (0°C and the second to high temperature, *i. e.*, 42°C for varying duration of time. Following the stress, the proportion of eggs that hatched was counted and emerging adults were collected daily.

Larva: For larval stress experiments, late 2nd or early 3rd instar larvae were collected. The experimental procedure was same as for eggs. After stress, the proportion of pupating larvae and the number of adults emerged were counted for each vial, each day.

Pupa: For pupal collection, forty pairs of adults oviposited for 24 h to minimize the age differences. Four days later, pieces of filter paper folded lengthwise were placed within the culture bottles for pupal collection. Pupae were collected every six hours and were stressed as above. The proportion of adults emerged was scored.

Adult: For adult thermo tolerance, we analyzed heat knockdown, heat survival, cold recovery and cold mortality as these are ecologically relevant and measurements were found repeatable across replicates as well as generations. Effects due to age, sex, anesthesia, ambient room temperature and thermal conditions of assay vials were controlled. Seven-day-old females were aspirated and introduced to assay vials which were pretreated at experimental temperatures for six hours so as to minimize the effects due to thermal variations.

Heat knockdown and heat survival were measured at 39 and 41°C. Individual flies were placed into 5 mL glass vials submerged into water bath set at constant experimental temperatures. Flies were scored for time (in minutes) taken to be knocked down. Survival was recorded by increasing

duration at experimental temperature until no fly survived. Chill coma recovery was measured by placing individual fly into empty 40 mL glass vial immersed in a 10% glycol solution cooled to a constant temperature of 0°C. Vials were removed from the cold bath after 8 h and recovery time of flies was scored at room temperature. This was measured as the time taken for a fly to stand upright. Chill coma mortality was measured by varying duration at cold bath until mortality reached 100%.

2.2.4 Elevated temperature experiment: To analyze the effect of 0.6°C increase in temperature till today, we used lower developmental threshold, 17°C and increased temperature by 0.6°C, 17.6°C for all experiments. Oviposition was done at 17°C, and then the eggs are divided into two groups for testing the effect of temperature increase on different developmental stages of *Z. indianus*.

2.3 Data analysis

For all traits measured, Statistica Release 5.0 (Statsoft Inc., Tulsa, OK, USA) was used for all calculations as well as illustrations. Geographical variations were analyzed by analysis of variance (ANOVA). For thermotolerance trait variability, data was subjected to three-way ANOVA. Mean trait values ($\pm SE$) were used for tabular data and illustrations. For significance level at $P < 0.05$, student's *t*-test and *P*-levels were used. Raw data were used to plot figures. For climatic data analysis, we used multiple regressions of trait values as a simultaneous function of T_{average} (T_{ave}) and relative humidity (RH) of the sites of origin of populations.

3 RESULTS

3.1 Temperature and distribution records of *Z. indianus* throughout a year

Data for minimum and maximum temperature throughout the year is shown in Fig. 1 (A). From a year long collection data from northern locality, Solan (30.58° N) during 2008 – 2009, it is concluded that *Z. indianus* (tropical species) was available during ten months, while *D. melanogaster* (cosmopolitan species) was caught in eleven months out of twelve (Fig. 1; B). *Z. indianus* was not recorded during the winter months but as soon as the weather becomes moderate they reappears.

3.2 Field cage study

To examine the overwintering survival under fluctuating field conditions, cages containing adults and eggs (1 – 2 h old) of two species, *D. melanogaster* and *Z. indianus*, were placed in

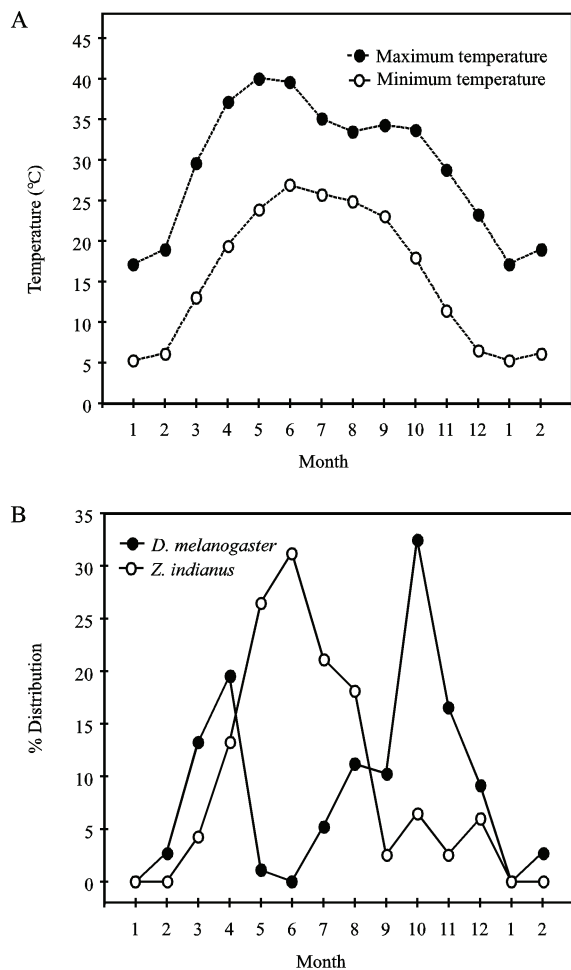


Fig. 1 Monthly minimum and maximum temperature (A) and monthly distribution (B) of a cosmopolitan, *Drosophila melanogaster* and a warm adapted *Zaprionus indianus* species of Drosophilidae
1: January; 2: February; 3: March; 4: April; 5: May; 6: June; 7: July; 8: August; 9: September; 10: October; 11: November; 12: December.

nursery and daily temperature was recorded and plotted against the days until flies are kept under field conditions (Fig. 2: A). Daily mortality and fecundity of the adult and hatchability and viability of eggs were recorded in field from December 15 (start of experiment) to February 15. For field cage experiments, *Z. indianus* was compared with *D. melanogaster*. Adult mortality patterns were almost same for both species (Fig. 2: B) suggesting that during extreme thermal conditions adults of both species are able to survive. Results showed that the mortality pattern of these two species was not affected by decrease in temperature. On the other hand, egg laying and hatching were ceased under extreme temperature for *Z. indianus* but not for *D. melanogaster*, although fecundity and hatchability were reduced (Fig. 2: C, D). In *Z. indianus*, eggs came out to be the dormant stages during winter months. We also tested the larvae and pupae for overwintering capacity. Egg dormancy of *Z. indianus* was induced by cold shock. About 75% of the eggs laid prior to lower temperatures were hatched when temperature became moderate, whereas viability of larvae and pupae were 3.12% and 17.01%, respectively. Between populations variability was assessed by ANOVA (Table 2). High variability in life history traits is due to longer developmental time of northern populations. There were significant ($P < 0.05$) line differences in all the three populations, suggesting genetic variation within populations.

3.3 Developmental thresholds of *Z. indianus*

Lower and upper developmental thresholds were calculated for *Z. indianus* and *D. melanogaster*. A clear pattern of eggs, larvae, pupae and total development was found. The upper thermal limits of

Table 2 Results of ANOVA applied to test variability between populations for lifetime fecundity, hatchability (%), viability (%), developmental duration and longevity of *Zaprionus indianus*

Source of variation	Population	Line	P × L *	Error
Traits	(df)	(2)	(19)	(1 080)
Fecundity (Lifetime)	% var	79.12	3.51	12.20
	P-level	0.0001	0.03	0.005
Hatchability (%)	% var	83.20	5.37	8.35
	P-level	0.001	0.05	0.001
Viability (%)	% var	81.57	6.96	6.84
	P-level	0.0005	0.05	0.003
Developmental duration	% var	77.86	5.77	8.27
	P-level	0.0007	0.04	0.007
Longevity (d)	% var	80.15	4.80	9.57
	P-level	0.0009	0.02	0.003

* P = Population; L = Line. The numeral in parentheses shows the degree of freedom for a variable.

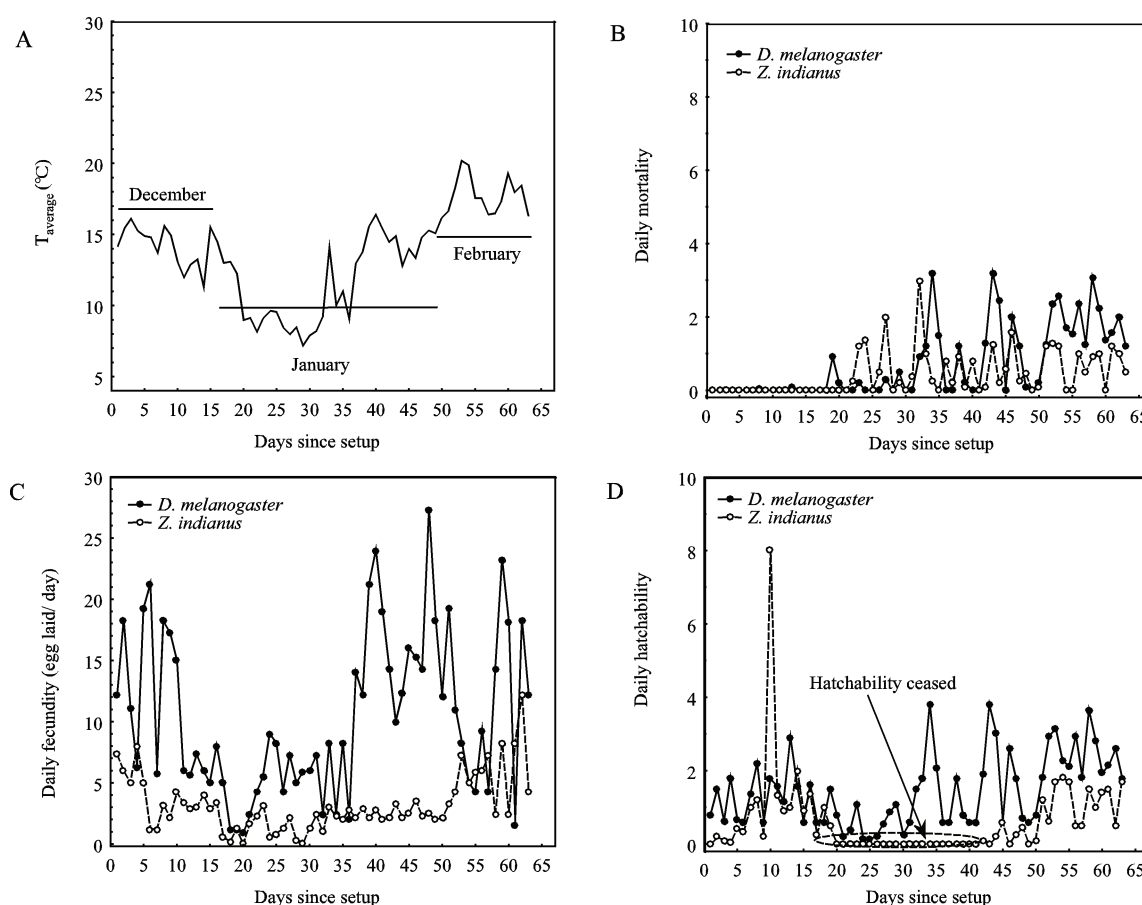


Fig. 2 Daily temperature records for the days of field cage set-up (A), daily mortality data for adults (B), daily fecundity (C), and daily egg hatchability (D) of *Drosophila melanogaster* and *Zaprionus indianus* under field conditions

Table 3 Average lower and upper developmental threshold temperature of two drosophilids from different biogeographical regions, *Zaprionus indianus* (tropical) and *Drosophila melanogaster* (temperate)

Developmental stage	<i>Z. indianus</i>		<i>D. melanogaster</i>		<i>P</i> -level	
	Lower ^a	Upper ^c	Lower ^b	Upper ^d	a vs. b	c vs. d
Egg	17.00 ± 0.10	33.00 ± 0.16	13.00 ± 0.19	31.00 ± 0.56	***	***
Larva	12.32 ± 0.11	34.00 ± 0.21	12.00 ± 0.34	32.00 ± 0.16	ns	**
Pupa	12.00 ± 0.51	35.50 ± 0.33	13.20 ± 0.51	32.50 ± 0.22	*	***
Egg-adult	17.00 ± 0.21	33.00 ± 0.12	13.20 ± 0.20	32.00 ± 0.14	***	**

* $P < 0.05$; ** $P < 0.01$; *** $P < 0.001$; ns: Non-significant.

the former are higher than the latter one (Table 3). The lower thermal limits for larval and pupal development were comparable, but the limit of egg hatchability differs significantly ($P = 0.005$). Egg-adult developmental threshold varies significantly. Upper developmental temperature for *Z. indianus* is higher than that of *D. melanogaster*, suggesting better adaptation towards warmer regions of the former.

3.4 Thermal resistance of *Z. indianus*: pre-adult and adult stages

In adult stage insects increase their survival through behavioral avoidance of stressful conditions thereby reducing the importance of physiological

plastic responses to changing environments. Eggs and pupae are immobile while larvae can crawl in fermenting fruits in which temperature may become high but due to high humidity conditions thermal variation in fruit is low that enable larvae to avoid more extreme temperatures.

Heat tolerance: After exposing all the pre-adult and adult life stages of *Z. indianus* to high temperature, pupae came out to be more tolerant stage to high temperatures, followed by adults. Larvae were most sensitive towards temperature. After an exposure of 90 minutes at 41°C, the pupal hatchability was 38%, adult survival 30%, whereas

eggs and larvae did not survive (Fig. 3). There was significant difference in adult knockdown and adult

survival after exposure to high temperature between geographical populations.

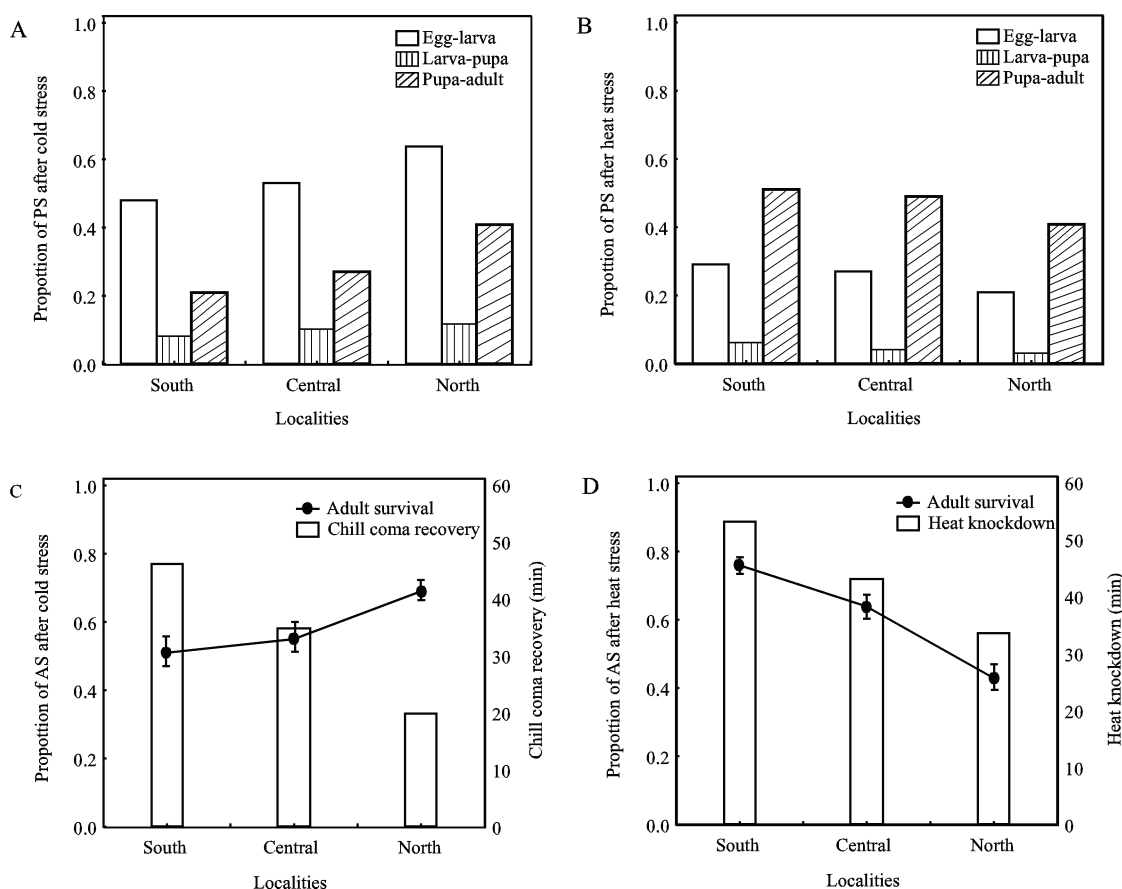


Fig. 3 Proportion of pre-adult survival (PS) after cold stress at 0°C for 24 h (A) and after heat stress at 41°C for 24 h (B), adult survival (AS) after cold stress (C) and heat stress (D)

Statistical differences were analyzed on the basis of *P*-values. Line plot in figure (C and D) shows chill coma recovery and heat knockdown (minutes), respectively.

Cold tolerance: Egg of *Z. indianus* was the most tolerant stage followed by pupal stage. Adults did not survive after 24 h and were the sensitive stage at low temperature. Chill coma recovery and chill coma mortality show significant variation due to population and temperature.

3.5 Variation in stress tolerance of *Z. indianus* and climatic association

Egg to adult survival of *Z. indianus* from south exceed that of north population at 39°C (data in min/h) while viability of the northern populations exceeds that of southern populations at 15°C. This relationship is different for pre-adult and adults of *Z. indianus*. Adult survival has been the most commonly studied character for measuring stress effects on fitness of drosophilids. But different life stages respond to thermal stresses in so many different ways that evolutionary consequences from periodic stress exposure cannot be predicted easily. We tested the genetic variation in survival/mortality

in offspring production after exposing different stages to high (42°C) and low (0°C) temperatures for different duration of time (Fig. 4). Difference among localities for total proportion of different heat stressed pre-adult stages that have reached the pupal and adult stages were significant. Pre-adult to adult developmental time varied significantly between temperature treatments.

In order to analyze the possible effects of local climatic conditions, we considered simultaneously two climatic factors, T_{ave} (average annual temperature) and RH_{cv} (coefficient of relative humidity). Regression analysis showed the effect of two climatic variables on thermotolerance traits and the data are given in Table 4. For all the four thermotolerance traits, we obtained significant values of coefficient of determination ($R^2 = 0.94 - 0.98$) when both the geographical parameters were considered together. The correlation coefficients were negative for heat knockdown and heat survival

but were positive for chill coma recovery and chill coma mortality. Fig. 5 indicates high physiological

tolerance towards high temperature of *Z. indianus* as compared to *D. melanogaster*.

Table 4 Multiple regression analyses of trait variability as a simultaneous function of coefficient of variation in mean monthly changes in temperature (T_{cv}) and relative humidity (RH) for three quantitative traits in latitudinal populations of *Zaprionus indianus* at 21°C

Traits	T_{cv}			RH		R^2
	$a \pm SE$	r_1	$b_1 \pm SE$	r_2	$b_2 \pm SE$	
Heat knockdown	63.21 ± 1.28	-0.57 ± 0.11	-0.59 ± 0.11	-0.43 ± 0.11	-0.47 ± 0.12	0.96
Heat survival	57.48 ± 1.84	-0.61 ± 0.10	-0.61 ± 0.13	-0.39 ± 0.13	-0.39 ± 0.10	0.94
Cold recovery	39.67 ± 2.08	0.59 ± 0.17	0.59 ± 0.12	0.41 ± 0.14	0.56 ± 0.14	0.98
Cold mortality	49.35 ± 2.92	0.53 ± 0.11	0.67 ± 0.13	0.47 ± 0.12	0.49 ± 0.12	0.97

$y = a + b_1x_1 + b_2x_2$ (where a = intercept; b_1 = slope for T_{cv} ; b_2 = slope for RH); r = correlation; R^2 = coefficient of genetic determination; SE = Standard error.

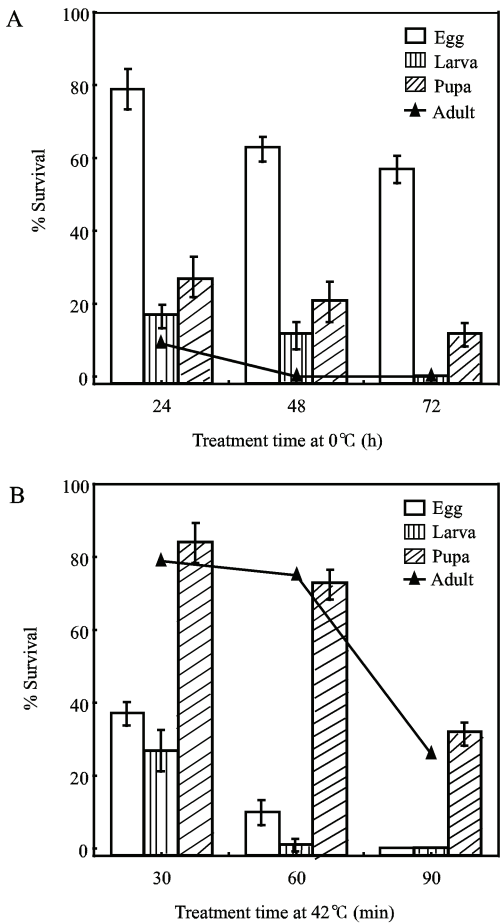


Fig. 4 Percent survival of pre-adult and adult stages of *Zaprionus indianus* at low (0°C) and high temperature (42°C) exposed to varying duration of time. Statistical differences were analyzed on the basis of P -values.

3.6 Effect of climate change on entry pathways of *Z. indianus*

Two different climate scenarios were considered; the lower developmental threshold for *Z. indianus*, *i. e.* 17°C and 17.6°C, as temperature has increased since last five decades till today is 0.6°C.

An increase of 0.6°C caused significant increment in population viability and a decrease in developmental duration (Table 5).

Table 5 Mean values for various life history traits at two temperatures

Traits	Temperature (°C)		t -test
	17.0	17.6	
Fecundity (Lifetime)	125 ± 2.32	203 ± 3.15	***
Hatchability (%)	34 ± 1.25	67 ± 2.31	**
Viability (%)	25 ± 1.95	59 ± 2.05	***
Developmental duration (d)	29 ± 2.33	21 ± 1.27	**

Values are mean of five replicates for each of the 20 isofemale lines. ** $P < 0.01$; *** $P < 0.001$.

4 DISCUSSION AND CONCLUSIONS

4.1 Invasion and establishment of *Z. indianus*

Z. indianus is considered the most common fly of the African continent (Lachaise and Tsacas, 1983). This species was first described by Gupta (1970) from India. Ectothermic insects face major challenges for adaptations to abiotic stresses; therefore, there is growing interest in the consequences of climate change on insect populations (Harrington and Stork, 1995). There are shifts in *Drosophila* species distributions as well as genetic changes in quantitative traits due to climatic warming. Widespread *Drosophila* species have shown that populations with increased levels of resistance evolve rapidly (Huey *et al.*, 2000). By contrast, the genetic potential of a specialist species to evolve in response to climate change is largely unknown. It is not known whether distributions of tropical and temperate species are limited by similar genetic attributes at the species borders. *Z. indianus* has been proven a successful invader in both tropical and

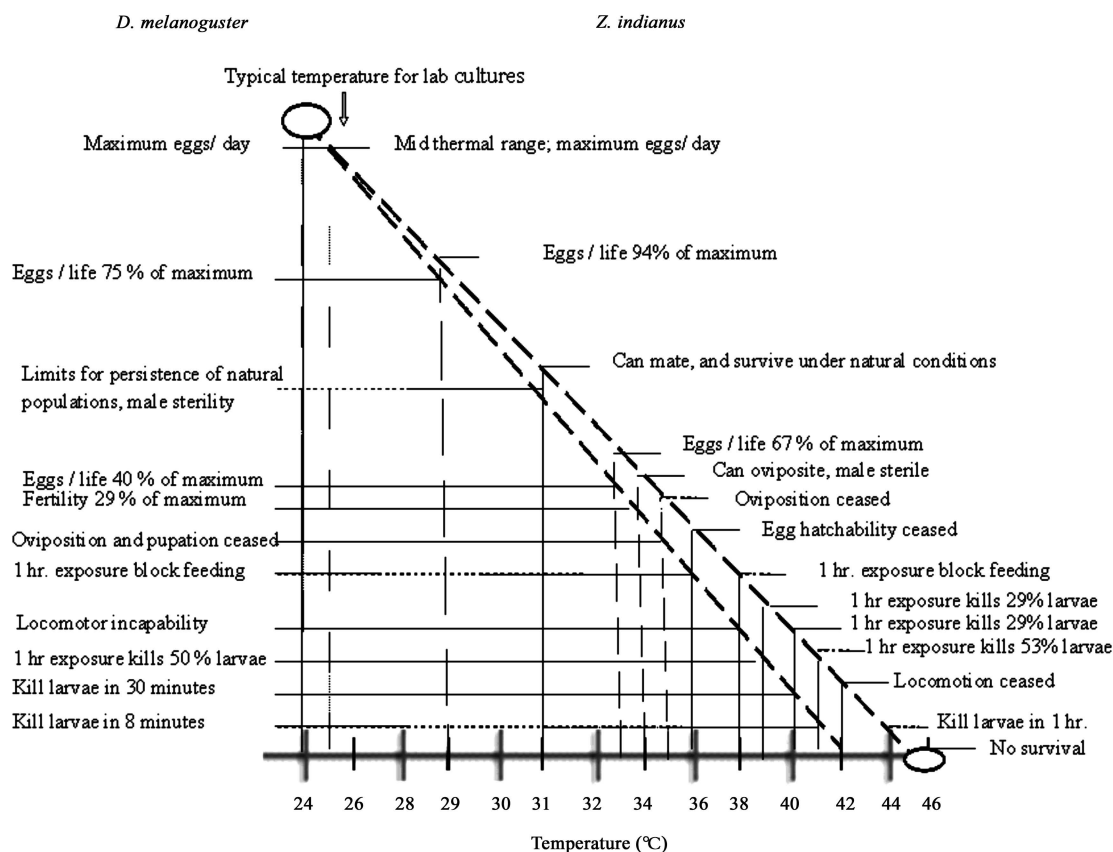


Fig. 5 Responses of cosmopolitan and tropical *Drosophila* species to high temperature
The right side of the figure indicates the generalist species – *D. melanogaster* and left side indicates warm specialist – *Z. indianus*.

temperate regions (Table 1). Expansion in tropical regions is easy to interpret as it is highly warm adapted, but the reasons for its invasion and establishment in temperate areas are largely unexplored. Studies have shown that with increase in latitude, lower developmental threshold decreases (Honek, 1996). Responses to temperature stress are well documented in temperate insects but information for tropical zone species is very little. Tropical (warm adapted) species have higher developmental thresholds than temperate (cold adapted) species (Trudgill and Perry, 1994). From the present study, the upper and lower developmental threshold differs significantly among one or two pre-adult stages. The complete development of *Z. indianus* occurs at 17°C (egg-adult) but if the eggs already hatch and if there is pupa formation prior to commence of low temperature, the development is not ceased, but of course there are increases in developmental duration and adult emergence. Differential pre-adult development towards lower developmental threshold is therefore an adaptation to low temperature. On the other hand, *Z. indianus* out competes *D. melanogaster* for the upper developmental threshold. Range of insect species is determined by temperatures that are required for efficient metabolism and reproductive success. Any

change in those temperatures, including those predicted to result from global warming, will result in range shuffling of these species. A further increase in lower temperature as a result of climate change will leads *Z. indianus* to further modify its thermal range.

4.2 Thermal resistance of *Z. indianus*: pre-adult and adult stages

Study of pre-adult survival is more ecologically relevant as they are immobile and face the stress directly, whereas, adults can escape stress by behavioral adaptations. The field cage experiments and laboratory study favor that *Z. indianus* overwinters in the egg stage and to a few extents as pupae. Eggs overwinter until the approach of warmer weather and remain in quiescent stage during winter months. The adult mortality data indicate that the ability to survive under low temperature conditions in the field is similar for *Z. indianus* and *D. melanogaster*. Egg hatchability data showed that egg went to quiescent period during low temperature conditions. Increase in cold hardiness in eggs of this species is the reason for successful overwintering and explains the outbreaks in summer. After comparing the cold tolerance of different geographical populations, we found that influence of climatic

factors of cold hardiness was significant. Also, significant variations in cold hardiness despite of similar climatic conditions imply a genetic basis. This was a part of genetic variation rather than plasticity. This study shows that the egg hatchability occurring in different populations is one of the reasons through which *Z. indianus* may adapt to cold.

Genetic variation in thermal sensitivity may differ among life stages of *Drosophila*. For three populations of *Z. indianus* whose survival after stress was measured from eggs, larvae, pupae and adults, the tolerance level among different stages was not related. Pupa was the most tolerant stage to high temperature and survived to those extreme temperatures that would kill other life stages, even adult, if exposed. Egg was the second most resistant stage, about 60% eggs developing to adults at stressful temperature of 42°C for varying duration of stress exposure. Larva was the most susceptible stage both at low and high temperature, showing maximum mortality. At both extremes larvae become black. Induced change in one life stage may continue to protect against stress in later stage. The difference in how pre-adult and adult stage behaves in thermotolerance may be correlated to climatic conditions which are known to different species (Parsons, 1977, 1979; Krebs and Loeschke, 1994a, 1994b; Krebs *et al.*, 1995).

Survival of eggs at one extreme and pupae at the other was higher as compared to adults exposed to same temperature and for same duration. The adults that survive after stress have reduced ability to offspring production (Krebs and Loeschke, 1994a, 1994b). We analyzed different traits in three populations and as can be seen from results presented in Table 3, there is a high genetic variability in various life history traits. We found clear between-population differences in stress tolerance and may be compared to climates of the sites of origin of populations. Climatic variables (T_{CV} and T_{ave}) are negatively correlated with latitude as well as with chill coma recovery and cold mortality. Heat knockdown and heat survival are positively correlated with climatic variables. There is a significant association between thermal tolerance traits and climatic variables. The population from higher latitudes was less resistant to heat but for cold they have high performance at all stages. Such differences at population level indicate that the genetic variation for thermotolerance traits is present in *Z. indianus*.

4.3 Climate change and entry of *Z. indianus*

Climate model projections summarized in the

latest IPCC report indicate that the global surface temperature has increased 0.6°C and is likely to rise a further 1.1 to 6.4°C (2.0 to 11.5°F) during the 21st century. Only a little increase of 0.6 degrees had resulted in change in all biological systems (Hughes, 2000; McCarthy *et al.*, 2001). According to Bale and Hayward (2010), insects that currently experience winter cryo-stress, increase in temperatures in the range of 1 – 5°C would increase survival. In the present study we mimicked these field conditions in laboratory by increasing lower threshold temperature (17 to 17.6°C). The results for both temperatures suggest that 0.6-degree increase in temperature is sufficient for *Z. indianus* to expand its boundaries as there was significant increase in all life history traits. The indication, therefore, is that a small increase in temperature in some way is permissive of growth efficiency.

4.4 Why one and not another?

Success and failure of an individual in an environment (old and/or new) depends on the ecological, genetic and physiological attributes. Individuals, which are able to function in a wide range of physical conditions and are having much genetic variability, have the invasion potential (Ehrlich, 1986). Many studies have shown that *Z. indianus* is a successful invader in many regions of the world (Table 1), but the factors governing its invasion are not still clear except a few studies about morphometrical traits associated with its invasion. This species has spread quite rapidly both through natural dispersal and via transportation. Our results show that this species has the great advantage of being able to survive under both hot and cold climates in spite of being warm adapted. Egg hardiness at lower temperatures might be the explanation of its occurrence at higher altitudes. Pre-adult stages of *Z. indianus* help to overcome both extremes of temperature. On the other hand, *D. melanogaster*, a cosmopolitan species that occurs in both tropical as well as temperate regions comes out to be less resistant to both heat and cold stress than *Z. indianus*. *D. melanogaster* escapes extreme conditions by behavioral escape (entering human constructions), whereas *Z. indianus* overcomes these conditions by physiological modifications in one or the other life stage.

4.5 Conclusions

Z. indianus, from the above results, has much genetic variability, is able to colonize both tropical and temperate regions and can function in a wide range of physical conditions. These qualities help this species to invade successfully, establish rapidly

and expand range at both borders. This species can cross the upper and lower barriers of thermal range easily by adapting in extreme environments via one or the other life stage.

ACKNOWLEDGEMENTS Department of Science and Technology, New Delhi is gratefully acknowledged for supporting the present research work through DST-INSPIRE Faculty fellowship (IFA-11LSBM-08).

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极端生理耐受性导致果蝇 *Zaprionus indianus* 在温带地区广泛分布

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摘要: 在过去的十年中, *Zaprionus indianus* 这一温带适应性果蝇已经入侵印度次大陆, 并扩大了其在该地区的分布。*Z. indianus* 能成功入侵是由于它具有很强的适应性和对极端生理条件的耐受性。对 *Z. indianus* (温带狭域分布种类) 和黑腹果蝇 *Drosophila melanogaster* (全球广域分布种类) 在极端温度下未成熟期和成虫期发育阈值的比较研究表明, 两者的死亡率和发育起点温度存在显著差异。为了检测越冬期间未成熟期和成虫期抗逆性和存活率的变化, 以采自印度温带和热带不同地点的 *Z. indianus* 种群进行饲养实验。在温带地区的田间养虫笼中以及恒定的实验室条件下监测这些种群的卵孵化率和成虫存活率, 直至全部成虫死亡。结果表明, 由于温带地区卵孵化率和存活率高, 导致总的孵化率和存活率在不同纬度间存在显著差异。卵至成虫发育实验结果表明, 低温条件下产下的卵在温度适中时成功发育成成虫。由此可见, 这种昆虫在未成熟期具有的气候适应性以及在成虫期具有的抗逆性可为该物种提供季节性保护。考虑到气候变暖情况, 即温度增加 0.6℃, 温度的少许改变都可能导致种群存活能力的显著增强和发育历期缩短。这些结果可解释 *Z. indianus* 为什么能够轻易突破障碍并适应新的环境。

关键词: *Zaprionus indianus*; 生命周期; 气候适应性; 生理耐受性; 气候变暖

中图分类号: Q969 **文献标志码:** A **文章编号:** 0454-6296(2012)11-1295-11

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